Landes Bioscience

www.landesbioscience.com

Supplemental Material to:

Jennifer McQueen, Dewald van Dyk, Barry Young, Chris Loewen and Vivien Measday

The Mck1 GSK-3 kinase inhibits the activity of Clb2-Cdk1 post-nuclear division

2012; 11(18)

http://dx.doi.org/10.4161/cc.21731

http://www.landesbioscience.com/journals/cc/article/21731

Figure S1

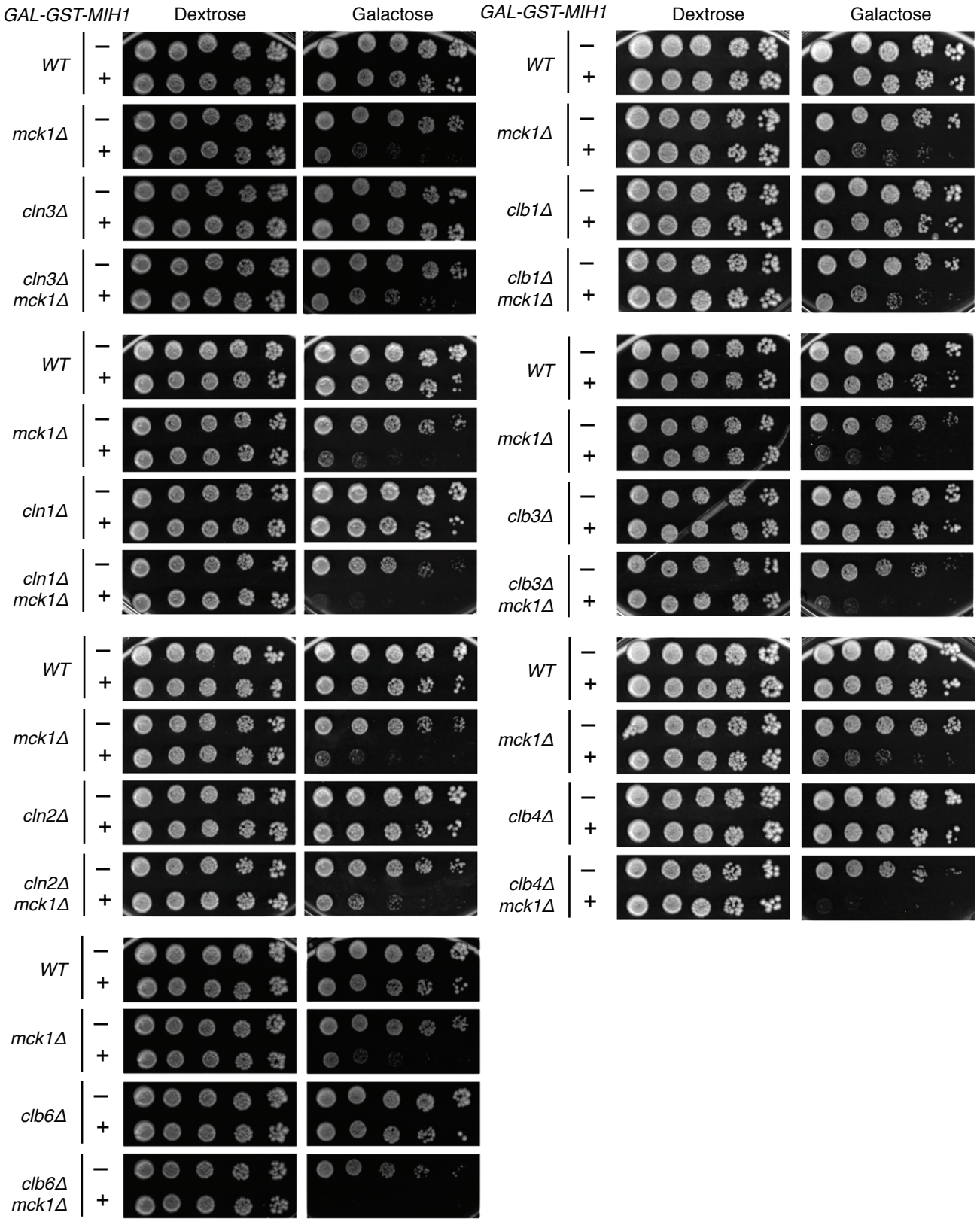
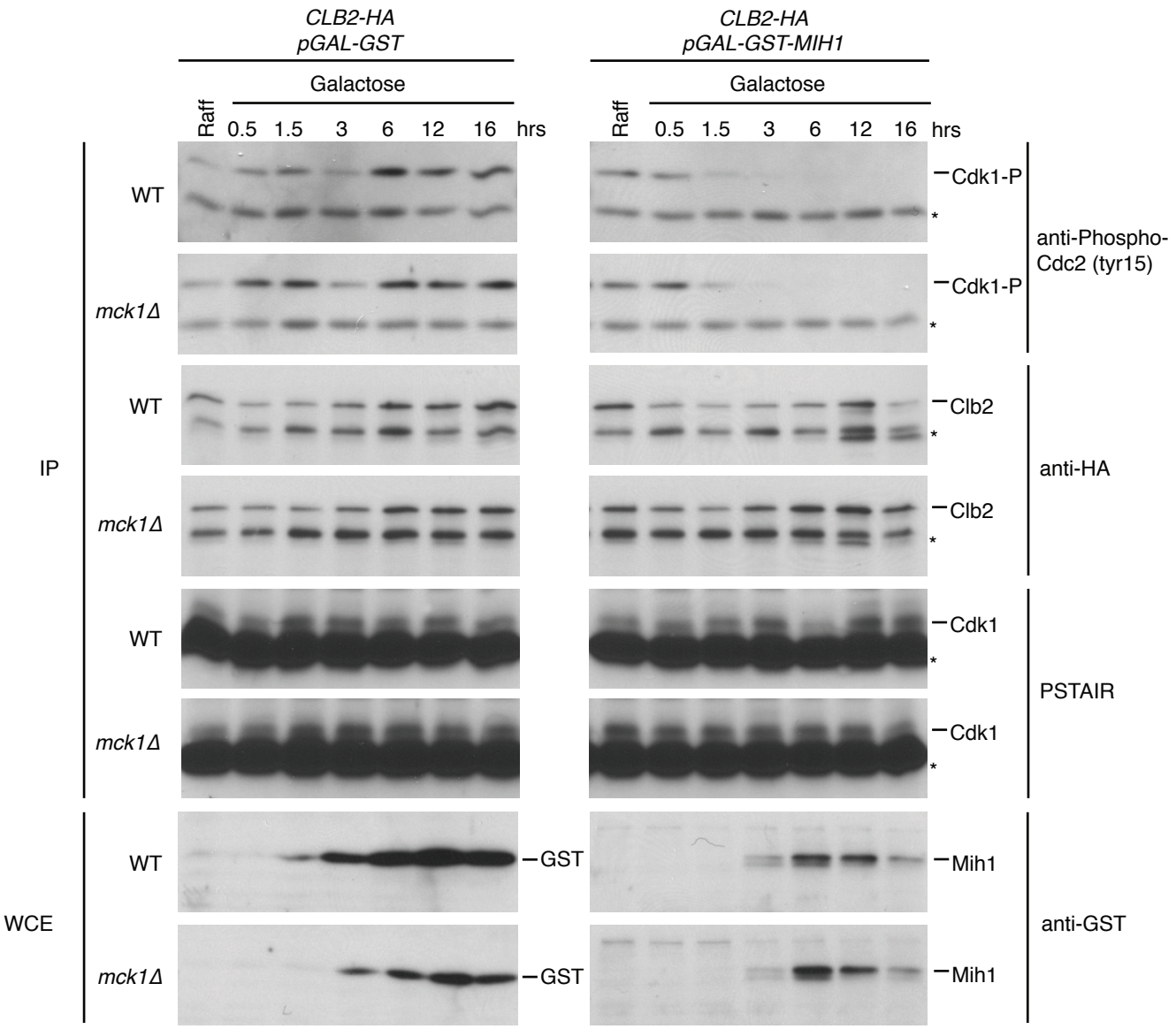

GAL-GST-MIH1
Dextrose
Galactose
WT
mck1Δ
cln3Δ
cln3Δ mck1Δ
clb1Δ
clb1Δ mck1Δ
cln1Δ
cln1Δ mck1Δ
clb3Δ
clb3Δ mck1Δ
cln2Δ
cln2Δ mck1Δ
clb4Δ
clb4Δ mck1Δ
clb6Δ
clb6Δ mck1Δ
–
+

Figure S2


CLB2-HA
pGAL-GST
CLB2-HA
pGAL-GST-MIH1
Galactose
Galactose
Raff
0.5 1.5 3 6 12 16 hrs
Raff
0.5 1.5 3 6 12 16 hrs
IP
WCE
WT
mck1Δ
WT
mck1Δ
WT
mck1Δ
WT
mck1Δ
Cdk1-P
Cdk1-P
Clb2
Clb2
Cdk1
Cdk1
Mih1
Mih1
GST
GST
anti-Phospho-Cdc2 (tyr15)
anti-HA
PSTAIR
anti-GST

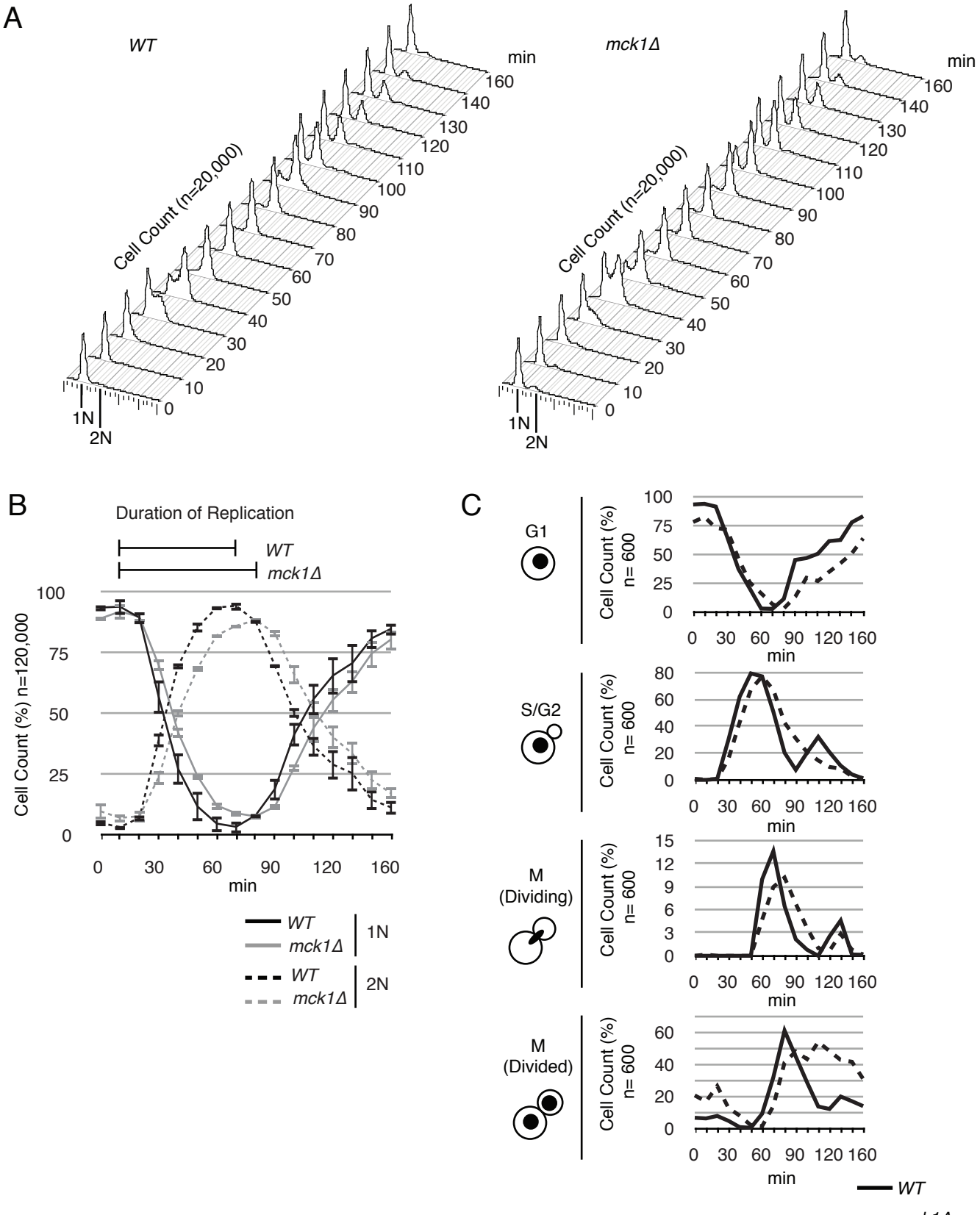

A
WT
mck1Δ
min
160
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Cell Count (n=20,000)
1N
2N
B
Duration of Replication
WT
mck1Δ
Cell Count (%) n=120,000
100
75
50
25
0
0 30 60 90 120 160
min
WT
mck1Δ
1N
WT
mck1Δ
2N
C
G1
S/G2
M
(Dividing)
M
(Divided)
Cell Count (%)
n= 600
100
75
50
25
0
80
60
40
20
15
12
9
6
3
0 30 60 90 120 160
min
WT
mck1Δ

Figure S4

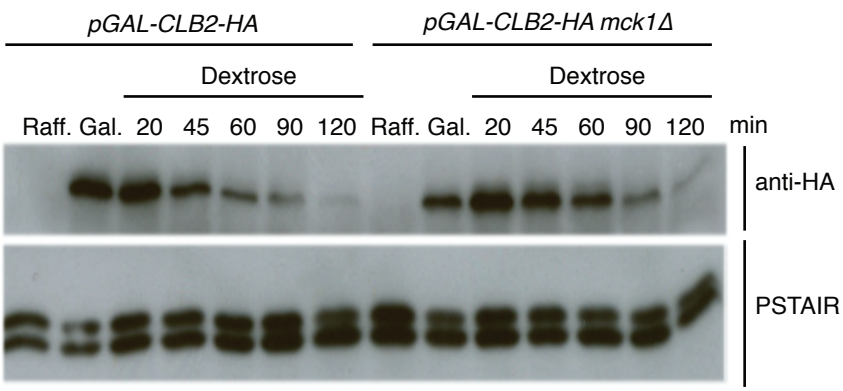
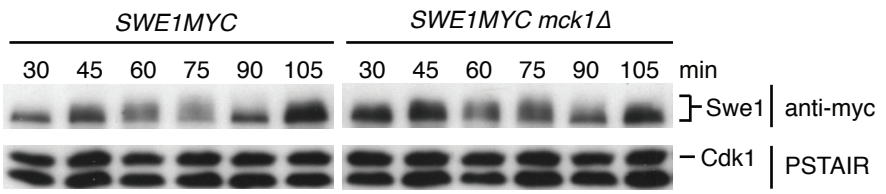
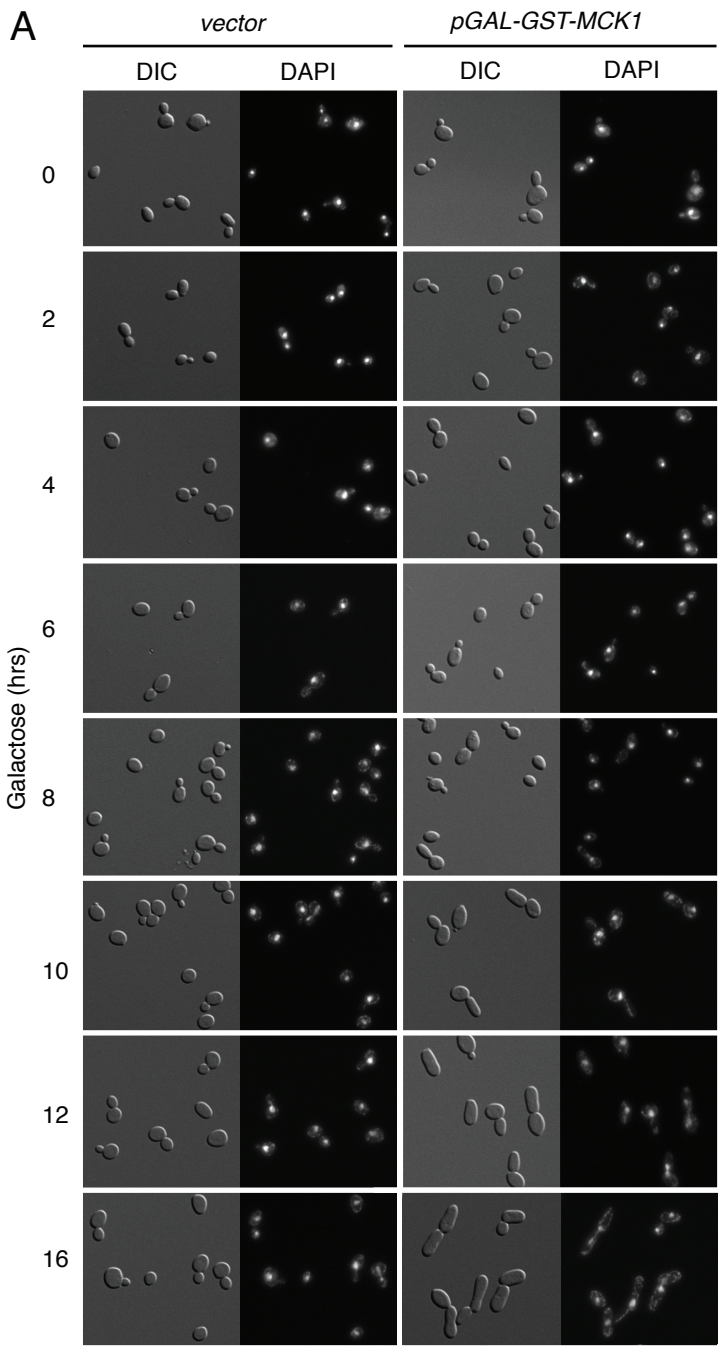
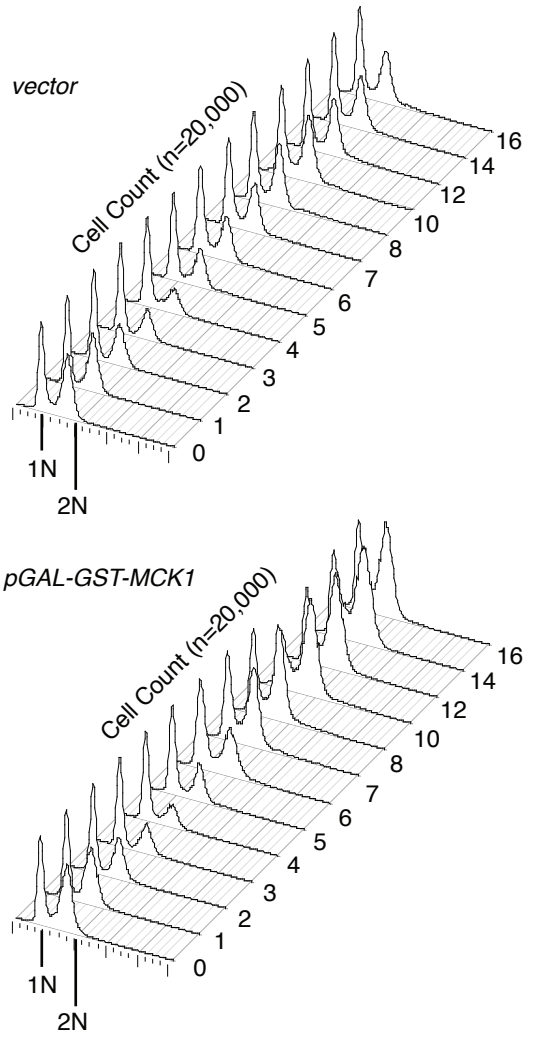
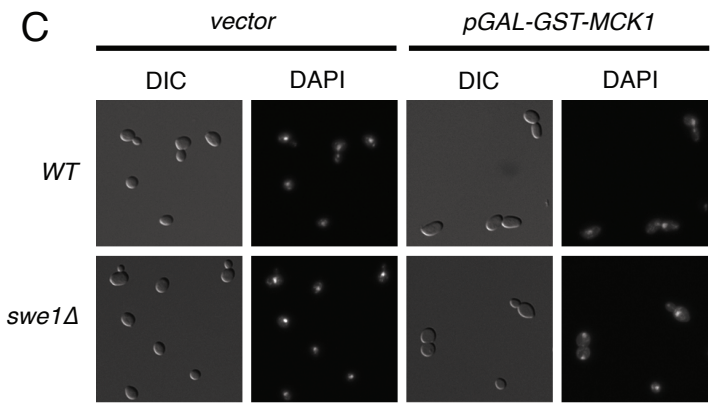

pGAL-CLB2-HA
pGAL-CLB2-HA mck1Δ
Dextrose
Dextrose
Raff. Gal. 20 45 60 90 120 Raff. Gal. 20 45 60 90 120 min
anti-HA
PSTAIR

Figure S5


SWE1MYC
SWE1MYC mck1Δ
30 45 60 75 90 105 30 45 60 75 90 105 min
Swe1
anti-myc
Cdk1
PSTAIR


A
vector
pGAL-GST-MCK1
DIC
DAPI
DIC
DAPI
0
2
4
6
8
10
12
16
Galactose (hrs)


B
vector
Cell Count (n=20,000)
16
14
12
10
8
7
6
5
4
3
2
1
0
1N
2N
pGAL-GST-MCK1
Cell Count (n=20,000)
16
14
12
10
8
7
6
5
4
3
2
1
0
1N
2N


C
vector
pGAL-GST-MCK1
DIC
DAPI
DIC
DAPI
WT
swe1Δ

Supplementary Figure Legends

Figure S1. Deletion of cyclins other than *CLB2* cannot rescue the toxicity of overexpressing *MIH1* in the *mck1Δ* strain. *pGAL-GST-MIH1* (+) or vector alone (-) were over expressed in wild type (WT), *mck1Δ*, *cyclinΔ* or *cyclinΔ mck1Δ* double mutants and plated onto dextrose and galactose media. Strains were grown to logarithmic phase, serially diluted onto the indicated media and grown for 4 days at 30˚C.

Figure S2. Mih1 is active in the absence of Mck1. The following strains were grown in SC^{-URA} raffinose (Raff) overnight and re-suspended in galactose media for the indicated times; *CLB2-HA pGAL-GST, CLB2-HA pGAL-GST mck1Δ, CLB2-HA pGAL-GST-MIH1, CLB2-HA pGAL-GST-MIH1 mck1Δ*. Clb2-HA was IP'd and analyzed by western blot with anti-phospho-Cdc2 (tyr15), anti-HA and anti-PSTAIR (Cdk1) antibodies. WCEs were analyzed with anti-GST antibodies. The asterisk (*) indicates background bands.

Figure S3. *mck1Δ* strains are delayed in DNA replication and cell cycle completion. WT and *mck1Δ* strains were synchronized in G1 with mating pheromone. After release from G1 arrest, cell cycle time points were taken every 10 minutes from two independent cultures in replicates of three and processed for FACS and microscopy. (A) A representative DNA content profile analyzed by FACS from one replicate of wild type (WT) and *mck1Δ* strains. (B) FACS data gated for 1N and 2N populations. Population numbers were averaged from the 6 samples taken (replicates of three from two

independent cultures) for each time point. Average population size as a percentage of total cells counted is shown. Error bars represent the standard deviation. Bars above the graph indicate the time required for replication. (C) Microscopy cell cycle analysis of budding index and nuclear division by DAPI staining was performed on duplicate samples from the same culture in both strains.

Figure S4. Clb2 degradation is unaltered in the absence of *MCK1*. Wild type and *mck1Δ* strains carrying *CLB2-HA* on a plasmid under the control of the *GAL1* promoter (*pGAL-CLB2-HA*) were grown to logarithmic phase in SC^{-URA} Raff. Cultures were washed and resuspended in SC^{-URA} galactose (Gal) for 30 minutes to induce *CLB2-HA* expression. Cultures were then washed and re-suspended in SC^{-URA} 2% dextrose to arrest *CLB2-HA* expression and samples were taken at indicated times (Dextrose). Clb2-HA degradation was followed by probing western blots with anti-HA antibody. Anti-PSTAIR (Cdk1) antibody was used a loading control.

Figure S5. Swe1 phosphorylation and degradation is unaltered in the absence of *MCK1*. *SWE1myc* and *SWE1myc mck1Δ* strains were synchronized with mating factor, released and Swe1-Myc protein levels monitored by western blot analysis every 15mins starting at 30 mins post mating factor release with anti-myc antibodies (Roche). Cdk1 (PSTAIR) is shown as a loading control.

Figure S6. Expression of *pGAL-GST-MCK1* causes bud elongation that is partially rescued by the deletion of *SWE1*. *pGAL-GST-MCK1* or vector alone were grown in wild type cells in SC^{-URA} Raff until early log phase then re-suspended into galactose media. Samples were taken for FACS and microscopy analysis every hour during galactose exposure until 8 hours and then again at 10, 12, 14 and 16 hours. (A) Representative microscopy images are shown for several time points. (B) FACs analysis representing DNA content profiles. (C) *pGAL-GST-MCK1,* or vector alone, was expressed in the presence or absence of *SWE1* for 16 hours. Representative microscopy images are presented.

Supplementary Tables

Table S1. Yeast strains used in study

Strain	Genotype	Source
YJM561	BY4742/S288C, MATα *ura3Δ::NAT^{R}can1Δ::STE2pr-Sp_HIS5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+*	[1]
YJM562	BY4742/S288C, *MATα YNL307CΔNatR can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+*	[1]
YM1761	YPH499/S288C, MATa *his3Δ200 ade2-101 ura3-52 lys2-801 leu2Δ1 trp1-Δ63*	Phil Hieter
YJM336	YPH499/S288C, MATa *his3Δ200 ade2-101 ura3-52 lys2-801 leu2Δ1 trp1-Δ63, mck1Δ::KanMX6*	This study
YJM881	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R cln3ΔkanMX*	This study
YJM879	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cln3ΔkanMX mck1ΔNAT^R*	This study
YJM945	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R cln1ΔkanMX*	This study
YJM947	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cln1ΔkanMX mck1ΔNAT^R*	This study
YJM949	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R cln2ΔkanMX*	This study
YJM951	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cln2ΔkanMX mck1ΔNAT^R*	This study
YJM875	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R clb6ΔkanMX*	This study
YJM877	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 clb6ΔkanMX mck1ΔNAT^R*	This study
YJM1087	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R clb1ΔkanMX*	This study
YJM1089	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 clb1ΔkanMX mck1ΔNAT^R*	This study
YJM892	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R clb2ΔkanMX*	This study
YJM887	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 clb2ΔkanMX mck1ΔNAT^R*	This study
YJM953	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R clb3ΔkanMX*	This study
YJM956	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 clb3ΔkanMX mck1ΔNAT^R*	This study
YJM1080	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ura3Δ::NAT^R clb4ΔkanMX*	This study
YJM1082	BY4741/S288C, MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 clb4ΔkanMX mck1ΔNAT^R*	This study
YJM97	S288C, MATa *his3Δ200 ade2-101 ura3-52 lys2-801 leu2Δ1 trp1-Δ63, swe1-13myc::HIS3*	This study
YJM99	S288C, MATa *his3Δ200 ade2-101 ura3-52 lys2-801 leu2Δ1 trp1-Δ63, swe1-13myc::HIS3, mck1ΔkanMX6*	This study
YJM871	W303, MATa *ura3Δ, trp1-1, ade2-1, his3-11, 15 Leu2-3, 112 can1-100 GAL+, Clb2-HA*	Mike Tyers

Strain	Genotype	Source
YJM1029	W303 MATa *ura3Δ, trp1-1, ade2-1, his3-11, 15 leu2-3, 112 can1-100, mck1ΔkanMX6 clb2-HA*	This study
YLM91	YPH499/S288C, MATa *his3Δ200 ade2-101 ura3-52 lys2-801 leu2Δ1 trp1-Δ63, MCK1-13myc::TRP1*	This study
YJM889	YPH499/S288C, MATa *his3Δ200 ade2-101 ura3-52 lys2-801 leu2Δ1 trp1-Δ63, MCK1-13myc::TRP1, mih1Δ::TRP1*	This study
YJM1143	YPH499/BY4742 S288C MATα *ade2-101 lys2-801 MCK1-13myc::TRP1 clb2ΔkanMX*	This study
YM2169 (DK186)	W303, MATa *leu2-3, 112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 GAL+ bar1*	Doug Kellogg
YJM1157	W303, MATa *leu2-3, 112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 GAL+ bar1 MCK1-13myc:KanMX6*	This study
YJM1243A	W303, MATa *leu2-3, 112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 GAL+ bar1 mck1D164A-13-myc:TRP*	This study
YJM1211A	W303, MATa *leu2-3, 112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 GAL+ bar1 CLB2-HA:HIS cdc28::cdc28-as1*	This study

Table S2. Plasmids used in this study.

Plasmid Name	Plasmid	Description	Reference or Source
BVM392	*pGAL-CLB2-HA*	*pWS945, CEN4, GAL10-CLB2-3xHA, LEU2, URA3*	[2]
BVM389	*Empty vector*	*pRS316, CEN6, URA3*	[3]
BVM311	*pGAL-GST-MIH1*	*pEGH, 2μ, GAL1/10-GST-6xHIS-MIH1, URA3*	Open biosystems
Not yet designated	*pGAL-GST-MCK1*	*pEGH,2μ, GAL1/10-GST-6xHIS-MCK1, URA3*	Open biosystems
BVM288	*Empty vector*	*pEGH, 2μ*	Open biosystems

References

1. Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Page N, et al. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 2001; 294:2364-8.
2. Seufert W, Futcher B, Jentsch S. Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. Nature 1995; 373:78-81.
3. Sikorski RS, Hieter P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 1989; 122:19-27.